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Salmonella enterica Control in Stick Carrots Through Incorporation of Coriander Seeds Essential Oil in Sustainable Washing Treatments

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Chemical disinfectants represent one of the commonly used practice in minimally processed vegetables food-chain. However, the scarce safety and sustainability of these agents force food industry to move toward more sustainable "green washing solutions." Among the latter, while the application of plant derivates for the control of several pathogens is already well-known, the potential anti-*Salmonella* activity of *Coriandrum sativum* seeds derivates is still unexplored and was therefore investigated in this study. In detail, Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of different coriander seed derivates (i.e., essential oil, hydrosol, and ethanolic extract) were determined by broth dilution against six *Salmonella enterica* strains isolated from fresh and minimally processed fruits and vegetables. Only the essential oil (EO) was effective *in vitro* with strain-dependent results. In addition, when mixed in co-culture, the strains were more sensitive to the essential oil treatment. Chemical investigations allowed to define (s)-(+)-linalool as major compound in the essential oil, and to underline interesting phenolic content with correlated antioxidant capacity. A cocktail of three strains of different serovars was selected and employed for a preliminary *in situ* trial on stick carrots. The obtained results allowed to establish that the application of coriander seed EO at concentrations of 5 μL mL^{-1} was able to reduce and contain the growth of the *Salmonella* cocktail up to 24 h at 10°C. Good sensory evaluation results were obtained by applying this EO concentration as washing treatment, especially in terms of color parameter. Further studies should be undertaken to emphasize the upstream activity, improving the formulation or exploiting a combined effect with other sanitizers or treatments (e.g., physical treatments). The present study contributes to the knowledge on coriander derivates activity against *Salmonella* spp. and on the potential application as sustainable washing treatment in removing this pathogen from fresh cut carrots.

Keywords: *Coriandrum sativum*, essential oil, anti-*Salmonella* activity, *Salmonella enterica*, Minimal processed vegetables, carrots, *in vitro* study, *in situ* study

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INTRODUCTION

Minimally processed vegetables (MPV) are defined as fresh, raw vegetables processed to supply ready-to-eat or ready-to-use foods (Nguyen-the and Carlin, 1994). These products can harbor a variety of spoilage microorganisms and a particular concern is related to the potential presence of cold-tolerant pathogens such as *Listeria monocytogenes, Escherichia coli, Bacillus cereus, Aeromonas hydrophila, Yersinia enterocolitica,* and *Salmonella* spp. Among them, *Salmonella* spp. represent a significant public health concern, being responsible of several foodborne outbreaks (Cai et al., 2016; Rossi et al., 2019). This food-borne pathogen, usually linked to poultry meat and products, is also found in a wide variety of fruits and vegetables (Pui et al., 2011) such as carrots (*Daucus carota*) (Gutiérrez-Alcántara et al., 2016; Liu et al., 2019) and fresh unpasteurized carrot juice (Torres-Vitela et al., 2013).

The major risks of *Salmonella* spp. contamination in the food industry are related to their occurrence in plants. In addition, strains belonging to this genus form biofilms on many food contact surfaces of processing equipment (e.g., cutting boards, knives) with consequent cross-contamination of vegetables (Lo Fo Wong et al., 2002; Doulgeraki et al., 2016). Effective intervention methods able to reduce *Salmonella* infections are based on washing and sanitization procedures, principally utilizing chemical disinfectants (e.g., chlorinated water, Silveira et al., 2017; Ramos et al., 2020). However, these chemical agents present several disadvantages in terms of safety and sustainability. Food industry should move away from these chemical disinfectants, therefore the implementation of washing steps with effective "green washing solutions" is of great importance (Gil et al., 2009).

Among these sustainable and innovative alternatives, essential oils (EOs), their distillation co-products (hydrosols) and extracts obtained from various plant materials have been investigated for their use in the food sector, since many of them have shown to possess antimicrobial, antioxidant and food preservative properties (Paparella et al., 2016; D'Amato et al., 2018), including interesting potentials in reducing *Salmonella* spp. on fruit and vegetables (Raybaudi-Massilia et al., 2006; Gündüz et al., 2009; da Silva et al., 2016; Rossi et al., 2019).

In one of our previous *in vitro* study (Pellegrini et al., 2018) thyme, savory, oregano and coriander seeds EOs obtained from Abruzzo territory cultivations, expressed an interesting anti-*Salmonella* activity. The majority of these EOs, however, can alter the taste of food or exceed acceptable flavor thresholds (Gutierrez et al., 2009; Dolati et al., 2016) due to strong balsamic notes of thymol (Calín-Sánchez et al., 2013, 2015). The high concentration of linalool (75%) in coriander seeds EO, on the other hand, may suggest mild aromatic characteristics more suitable for food application.

Research reports on composition, antioxidant and antimicrobial activity of *Coriandrum sativum* seeds extracts are limited, especially concerning food processing applications. Thus, the aims of the present work were to (i) enrich the knowledge regarding *in vitro* anti-*Salmonella* activity of coriander seeds derivates and to (ii) investigate their potential application as washing treatment on stick carrots MPV.

MATERIALS AND METHODS

Plant Material Extraction

C. sativum seeds, retrieved from a local farmer in Abruzzo, were subjected to different extraction procedures to obtain coriander seed EO (CEO), hydrosol (CH), and extract (CX).

The CEO was steam-distilled from the whole seeds (1 kg) without any further processing, by means of a E0105 12 lt PLUS Essential Oils Extractor (Albrigi Luigi Srl, Italy) for 2 h. After distillation, CEO was transferred to an amber glass vial with anhydrous sodium sulfate (Sigma Aldrich, USA), conditioned with argon and sealed. The CH was recovered from the same extraction process (i.e., remaining aqueous fraction after EO removal) and transferred to sterile tubes. Both collected CEO and CH were stored under refrigeration at 4°C. The distillation was conducted in triplicate.

The CX was obtained through rapid solid-liquid dynamic extraction (RSLDE) performed by means of 500 mL Naviglio extractor® (Atlas Filtri, Padua, Italy). Ten hundred g of the matrix were subjected to the RSLDE process with 250 mL of absolute ethanol (using a reduction chamber unit) as follows: 30 cycles (with a maximum pressure of 8 bar), each cycle composed by 12 hits in the dynamic phase (2 min duration) and a duration of the static phase of 2 min. At the end of the extraction process, the ethanol extract was filtered, collected in a pear-shaped evaporation flask and the solvent was removed by means of the Laborota 4,000 rotary evaporator (Heidolph–Schwabach, Germany). The obtained CX was transferred to an amber glass vial and stored under refrigeration at 4°C. The extraction was performed three times.

Microbial Strains and Growth Conditions

Six *Salmonella enterica* of different serovars were used in the study: *S.* Derby, *S.* Thompson, *S.* Napoli and *S.* Typhimurium monophasic variant, *S.* Kasenyi and *S.* Veneziana were isolated from fresh and minimally processed fruits and vegetables as previously described (Losio et al., 2015; Rossi et al., 2019). *Salmonella* strains were obtained from stock cultures maintained at −80°C in cryovials in 20% v/v glycerol/tryptic soy broth (TSB, Oxoid Thermofisher, Rodano. Italy). All strains were cultivated routinely on TSB at 37°C and stored at 4°C on tryptic soy agar slants.

Determination of Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

C. sativum derivatives were studied for their MIC values according to the microdilution method, as described by CLSI guidelines (Clinical and Laboratory Standards Institute, 2011). The CEO was dissolved in sterile PBS (Phosphate Buffer Saline) 50 mM pH 7.0 to reach the initial concentration of 40 μL mL^{-1}, as already described (Mazzarrino et al., 2015). The hydrosol was used as it stands, while the evaporated RSLDE extract was diluted with distilled water (40 mg mL^{-1}). The inocula were prepared

from overnight broth cultures (early stationary growth phase), and suspensions were adjusted to the required microbial load (5×10^5 CFU mL^{-1}). A positive (100 μL of TSB plus 100 μL inoculum) and a negative control (200 μL of sterile TSB) were considered for each strain. The MIC value was considered as the lowest EO concentration that prevented growth after 48 h of incubation at 37°C. The MBC, the lowest concentration of the essential oil at which incubated microorganisms are completely killed, were confirmed by re-inoculating on agar plates with 10 μL of each culture medium from the microplates.

MBCs and further investigations were carried out only on CEO, as for the other derivates not satisfying MIC values were recorded. These assays were performed for all six strains in single culture and for some strains in co-culture.

According to MIC/MBC results, the 2 most sensitive serovars were selected and coupled with two other serovars. The cocktails were constituted as follows: cocktail 1 (C1): *S*. Thompson, *S*. Derby and *S*. Napoli; cocktail 2 (C2): *S*. Thompson, *S*. Derby and *S*. Typhimurium. All the analyses were performed in triplicate.

Determination of Inactivation/Growth Kinetics

A turbidimetric analysis was conducted to evaluate inactivation/growth kinetics of the strains in presence of different CEO concentrations. Inocula were prepared as described in section Determination of Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) and incubated at 37°C. The plates were automatically scanned and recorded every 15 min for 2 days by the OmniLog incubator/reader (Biolog Inc., Hayward, USA). The scanning technology used by Omnilog records the color change in the well as digital Omnilog units. Data were recorded using the OmniLog PM software provided by the same company and then exported into Microsoft Excel Professional for further data processing. The values were expressed as mean of replicated growth curves. The experimental data were fitted according to the equation of Baranyi and Roberts (1994), by means of DMFit software (https://browser.combase.cc/DMFit.aspx) to estimate growth parameters such as lag phase length and maximum growth value, as previously described (Mazzarrino et al., 2015).

Chemical Analyses of CEO

The CEO was investigated for its chemical composition through GC-MS analysis, following the procedure previously described by Pellegrini et al. (2018).

The antioxidant capacity (TEAC/ABTS, DPPH, FRAP) and total phenolic content (TPC) were also investigated.

The TEAC/ABTS assay was determined as described by Masaldan and Iyer (2011). The TEAC/ABTS results of the samples were estimated in terms of mg Trolox equivalent (TE)/g EO as the mean of three replicates.

The FRAP was determined by using the potassium ferricyanide-ferric chloride method described by Oyaizu (1986). The FRAP of the samples was estimated in terms of mg Trolox equivalent (TE)/g EO as the mean of three replicates.

The DPPH radical-scavenging activity of the EOs methanolic solutions was measured according to the method described by Brand-Williams et al. (1995). Results were expressed in terms of mg Trolox equivalent (TE)/g EO as the mean of three replicates.

The TPC was determined by the Folin–Ciocâlteu method described by El-Lateef Gharib and Teixeira da Silva (2013). The TPC results were expressed in terms of mg Gallic acid equivalents (GAE)/g EO as the mean of three replicates.

Study of CEO Activity on Stick Carrots

Fresh carrots (*Daucus carota* L.) were purchased at a local market and those with defects were discarded. Selected carrots were washed to reduce the native spoiling flora as reported by Martínez-Hernández et al. (2017); then, the carrot samples were manually peeled by a hand potato peeler and cut into 4 × 0.3 cm sticks.

Three *Salmonella* serovars were used in the study as a cocktail. In particular, the inoculum of *S*. Thompson, *S*. Derby and *S*. Napoli was standardized in PBS at about 5×10^5 CFU mL^{-1}. The carrot samples were aseptically submerged into *Salmonella* suspension for 30 min at room temperature (Ruiz-Cruz et al., 2007) and stand to dry 30 min in a class II biological safety cabinet. CEO was diluted in sterile PBS as described in section Determination of Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) to reach the final concentration of 5 μL mL^{-1}. The carrot samples were aseptically transferred into an open sterile glass jar, containing the CEO solution. Also control samples were included, dipping the carrot samples in the same way in PBS alone. All samples were dipped into the appropriate solution for 2 min, then the excess solution was drained off and samples were air-dried for 30 min in a biological safety cabinet (Gonzalez et al., 2004). Carrots sticks were aseptically placed in polyethylene bags to prevent water loss and stored under aerobic conditions for 48 h at 10°C (abuse temperature).

The samples (25 g) were aseptically transferred in stomacher bags containing 225 mL of sterile saline solution, homogenized in stomacher (Lab Blender 400, Seward Medical, UK) for 5 min, then serial decimal dilutions were prepared in sterile saline solution. *Salmonella* counts were determined using Xilose Lysine Deoxicholate agar–XLD (Oxoid–Rodano, Italy), after incubation at 37°C for 24 h. The analyses were carried out in duplicate at the following intervals: 0, 1, 24, 48 h, considering time 1 h as time after the application of the treatment. To assess the absence of *Salmonella* spp. in non-inoculated stick carrots, control samples were included and followed for the same time of storage.

Sensory Evaluation

Sensory evaluation was performed as proposed by Cui et al. (2017). 30 people (15 males and 15 females) aged 26–60 years were recruited from the staff of the University of Teramo. Control and treated carrot sticks were served at room temperature separately. Unsalted crackers and mineral water (room temperature) were provided to clean the palate between samples. The hedonic scale consisted of 9 levels (1: dislike extremely/ extremely bland and 9: like extremely/extremely flavorful), in which the panelists evaluated the different attributes (i.e., color, aroma, taste, and overall acceptability).

Statistical Analysis

Microbiological counts were converted to Log CFU g^{-1} and all results were expressed as mean ± standard deviation. Data were subjected to analysis of variance (ANOVA) and pair-comparison with the same group was achieved applying Tukey's *post-hoc* test procedure at $p < 0.05$. Correlations between TPC and AOC results were calculated by means of Pearson Correlation. Statistical tests were carried out using XLSTAT 2017 (Addinsoft, Paris, France).

RESULTS

Determination of Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The *C.sativum* seeds extracts were tested for their antimicrobial activity against six *S. enterica* strains of different serovars, previously isolated from vegetables, and belonging to the most recently isolated *Salmonella* serotypes in Italy in this kind of products (Rossi et al., 2019).

The results obtained from MIC/MBC assay were reported in **Table 1**. As showed, among the tested extracts, the CEO was the only one able to exert an antibacterial activity against *S. enterica* serovars, with a strain-dependent sensitivity. Indeed, *S.* Typhimurium and *S.* Kasenyi showed high resistance to treatment with CEO, whose MIC and MBC values (μL mL^{-1}) were higher than the maximum concentration employed in the analysis (20 μL mL^{-1}). Otherwise, the most sensitive strains were *S.* Derby and *S.* Thompson, for which the effect was both bacteriostatic and bactericidal at a concentration of 5 μL mL^{-1}. The essential oil effectiveness was also observed for *S.* Napoli and *S.* Veneziana, however the inhibitory concentrations of CEO for the two strains were 20 μL mL^{-1}.

In order to employ a cocktail consisting of 3 *Salmonella* strains for an *in situ* study, the evaluation of the MIC and MBC of co-cultured microorganisms was performed. Specifically, following the MIC/MBC results, the two most CEO sensitive serovars (*S.* Derby and *S.* Thompson) were selected and both *S.* Napoli and *S.* Typhimurium were tested as additional strains.

Table 1 shows also the results of the MIC and MBC values of coriander EO on *Salmonella* strains co-cultures. The comparison clearly shows that *Salmonella* serovars inoculated in ternary cultures, are more sensitive to the treatment with CEO. *S.* Napoli in fact, was individually inhibited at the 20 μL mL^{-1} CEO concentration, nevertheless, when co-cultured in presence of *S.* Thompson and Derby (Cocktail 1) a concentration of 5 μL mL^{-1} was able to exert bactericidal effect. A similar behavior was observed for *S.* Typhimurium; while in single culture, a CEO concentration >20 μL mL^{-1} was requested for the inhibition, only 10 μL mL^{-1} concentration were sufficient to inhibit C2 co-culture. According to these results, C1 co-culture (*S.* Napoli + *S.* Thompson + *S.* Derby) was selected for *in situ* testing.

TABLE 1 | Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Coriandrum sativum* extracts against single strains and cocktails of *Salmonella enterica*.

Salmonella enterica	CEO		CH	CX
	MIC (μL mL^{-1})	MBC (μL mL^{-1})	MIC (μL mL^{-1})	MIC (mg mL^{-1})
S. Napoli	20	20	>500	>20
S. Veneziana	20	20	>500	>20
S. Derby	5	5	>500	>20
S. Typhimurium var. monof.	>20	>20	>500	>20
S. Thompson	5	5	>500	>20
S. Kasenyi	>20	>20	>500	>20
Cocktail 1	5	5	–	–
Cocktail 2	10	10	–	–

CEO, Coriandrum sativum seeds essential oil; CH, Coriandrum sativum seeds hydrolate; CX, Coriandrum sativum seeds extract. Cocktail 1, S. Napoli + S. Thompson + S. Derby; Cocktail 2, S. Typhimurium var. monof + S. Thompson + S. Derby.

Determination of Inactivation/Growth Kinetics

In **Figures 1A,B** the inactivation/growth kinetics of the two assayed cocktails (C1 and C2, respectively) were presented.

Regarding C1, as displayed in **Figure 1A**, from the wells where different concentrations of CEO were present, different kinetics with respect to the control (C1) were recorded. In particular, at 10 and 5 μL mL^{-1} no growth was observed along the 48 h of analysis (lethal concentrations); at 2.5 μL mL^{-1} concentrations, a Lag phase extension was detected (22 h with respect to 1.1 hour observed for control). In presence of 1.25 μL mL^{-1}, after a slight initial Lag phase elongation of about 4 h, a subsequent increase of microbial growth was detected, being the maximum growth value of 87.4 Omnilog Units in spite of 64.8 Omnilog Units observed for C1, according to the data modeling.

In reference to C2 (**Figure 1B**), only for 10 μL mL^{-1} a lethal effect was observed. The Lag phase extension was greater at concentration of 5 μL mL^{-1} (21.8 h), followed by 2.5 μL mL^{-1} (9.6 h); both concentrations also determined lower maximum growth values (59 and 61.5 Omnilog Units respectively) than the control (86 Omnilog Units), confirming the antimicrobial action of CEO at those concentrations. On the contrary, 1.25 μL mL^{-1} CEO concentration seemed to exert very slight effects on the C2 cocktail growth; its recorded kinetic in fact, was almost similar to the control one, with a sharp increase in the first hours of incubation and a similar final growth value.

Chemical Analyses of CEO

Table 2 reports the chromatographic characterization by GC-MS of CEO, which allowed to identify the 95% of the volatile mixture. From the obtained results, it was underlined that CEO was constituted essentially by monoterpenes. In particular, (S)-(+)-linalool accounted for the 78% of the total volatile mixture; the remaining 17% of identified compounds was composed only by five other monoterpenes, with a major contribution of trans-β-ocimene, *cis*-geraniol, and camphor.

The antioxidant activity assays demonstrated interesting results (**Table 3**), in terms of radical scavenging activity (TEAC/ABTS and DPPH) and ferric reducing antioxidant power

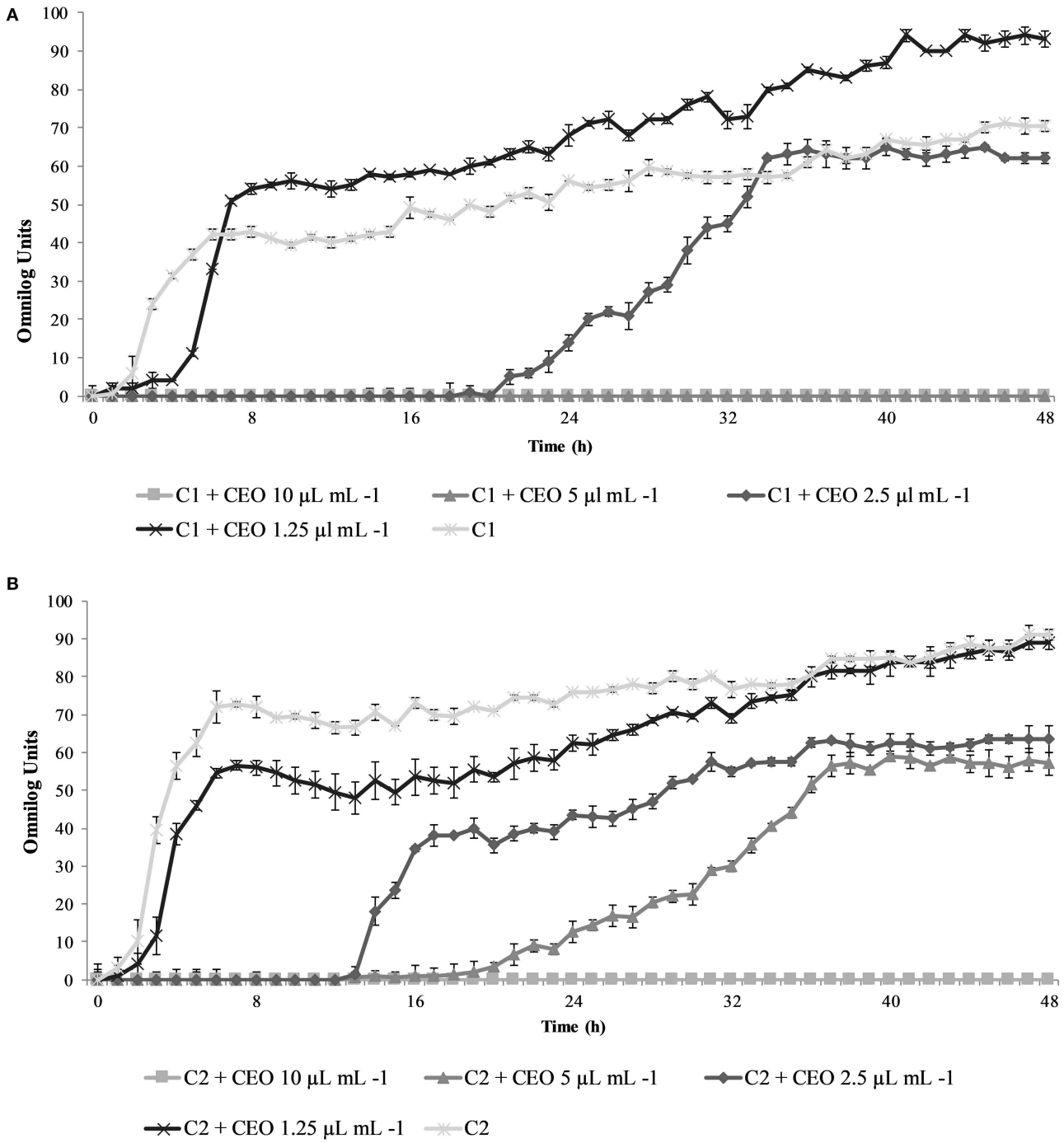

FIGURE 1 | Growth/inhibition dynamics of *Salmonella* strains in co-cultures, in presence of different concentrations of *Coriandrum sativum* seeds essential oil (µL mL^{-1}) during 48 h of incubation at 37°C. **(A)** *S*. Thompson, *S*. Derby and *S*. Napoli co-colture (C1); **(B)** *S*. Thompson, *S*. Derby and *S*. Typhimurium co-culture (C2).

(FRAP). These results were also strongly positively correlated ($r > 0.9$; $p < 0.05$) with total phenolic content.

Study of CEO Bio-Preservative Activity on Stick Carrots

The results of the preliminary study about CEO anti-*Salmonella* activity on stick carrots are displayed in **Figure 2**. First of all, a slight "matrix effect" was recorded: in fact, at time 1, the not treated inoculated samples (C), showed a difference in microbial load of about 0.8 Log with respect to the inoculum. This effect could be related to the specific complexity of food matrix, that interfere with bacterial cells. Furthermore, in untreated control samples (C), the counts of *Salmonella* were almost stable during the storage time.

TABLE 2 | Gas Chromatography-Mass Spectrometry (GC-MS) characterization of *Coriandrum sativum* seeds essential oil.

ID	RID	RIE	Relative abundance (%)
α-Pinene	939	927	0.25 ± 0.06
δ-3-Carene	998	1,001	0.89 ± 0.02
trans-β-Ocimene	1,015	1,033	4.58 ± 0.58
(s)-(+)-Linalool	1,100	1,079	78.48 ± 0.11
Camphor	1,139	1,132	4.44 ± 0.76
cis-Geraniol	1,254	1,253	4.77 ± 0.11
Humulene	1,467	1,412	1.83 ± 0.10
Total identified compounds			95.24 ± 0.01

ID, component name; RID, retention index retrieved from http://webbook.nist.gov/chemistry/ for the same analysis conditions; RIE, experimental retention index referred to C8–C40 n-alkane mixture standard. Results are expressed as relative abundance ± standard deviation (n = 3).

TABLE 3 | *Coriandrum sativum* seeds essential oil: Total Phenolic Content (TPC) estimation, by Folin-Ciocâlteu method, and Antioxidant Activity Capacity (AOC) assays by Trolox Equivalent Antioxidant Capacity with 2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (TEAC/ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP).

TPC (mg GAE/g OE)	AOC (mg TE/g OE)		
	TEAC/ABTS	DPPH	FRAP
5.33 ± 0.03	2.55 ± 0.40	0.53 ± 0.01	36.37 ± 0.37
Pearson correlation coefficients			
	0.991	0.967	0.985

TABLE 4 | Sensory evaluation results obtained for stick carrots treated with *Coriandrum sativum* seeds essential oil washing treatment and for control stick carrots.

Sensory parameter	Control	Treated
Aroma	7.2 ± 0.5 a	7.5 ± 0.1 a
Taste	8.5 ± 0.2 a	7.9 ± 0.5 a
Color	6.0 ± 0.5 b	8.0 ± 0.1 a
Overall acceptability	7.8 ± 0.5 a	7.5 ± 0.2 a

For the same parameter (same row), results followed by the same case-letter are not significantly different according to Tukey' HSD post hoc test (p > 0.05).

From **Figure 2** it is also possible to infer how immediately after performing the treatment with the essential oil (1 h), the application of CEO on carrots determined a significant reduction ($p < 0.05$) in *S. enterica* of about 1 Log CFU g^{-1}. After 24 h, the difference between treated samples and control samples still persist, while after 2 days this reduction was lost, with counts comparable to those of the control.

The analysis of uninoculated carrots revealed the absence of *Salmonella* spp.

Sensory evaluation results were reported in **Table 4**. As can be observed, any negative alteration was observed among treated and untreated samples in terms of aroma, taste and overall acceptability. For these parameters no significant differences ($p > 0.05$) were recorded between the investigated samples. Color was positively affected by CEO washing treatment, obtaining higher scores ($p < 0.05$) with respect to the control.

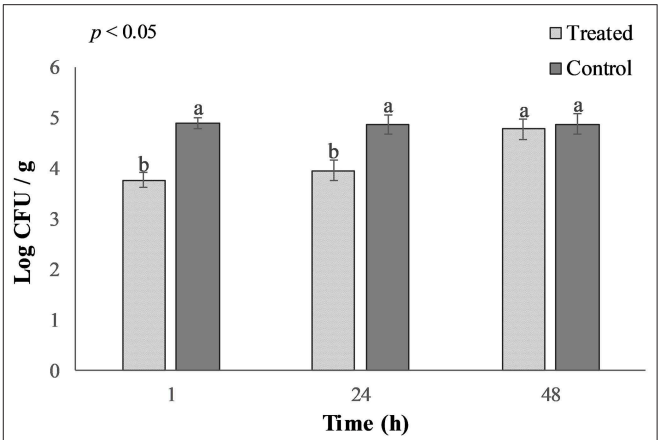

FIGURE 2 | Evolution of *Salmonella* counts during refrigerated storage of stick carrots (48 h, 10°C) samples treated with 5 μL mL^{-1} *Coriandrum sativum* seeds essential oil and in control ones. For the same assay results followed by the same case-letter are not significantly different according to Tukey' HSD *post hoc* test ($p > 0.05$).

DISCUSSION

The results of the present study showed that, among the *C. sativum* seeds derivatives, the only effective, at the tested concentrations, was the essential oil.

This better anti-*Salmonella* activity could be due to the chemical compounds contained in the essential oil. As reported in literature, (s)-(+)-Linalool, the major chemical component of CEO, has a remarkable antimicrobial activity (Aelenei et al., 2019), but the antimicrobial effect of the essential oil was probably due not only to its contribution. In fact, it is well-known that different biological activities of plant derivatives can be a function of synergistic interactions among major and minor compounds (Moon et al., 2006; Lima Oliveira et al., 2018). In example, camphor is present in other herbs and essential oils (basil, marjorane, rosemary, sage), and it is known for its antimicrobial activity, related to the ability to disturb the membrane phospholipid bilayer and to interact with enzyme and proteins (Bouazama et al., 2017). Its presence in low percentages in the studied coriander essential oils could boost (s)-(+)-linalool activity, still remaining within the safety daily doses established by EFSA (2008).

The chemical composition results obtained for the CEO were in accordance with literature data (Lo Cantore et al., 2004; Mandal and Mandal, 2015). A similar chemical composition was also obtained in our previous study (Pellegrini et al., 2018) for an essential oil recovered from coriander seeds purchased from the same local producer, but belonging to a different seeds batch. A different situation emerged when antioxidant activity and total phenolic content results were compared with those

obtained in this previous study (Pellegrini et al., 2018). In fact, the essential oil recovered from the new batch of seeds gave better results. In fact, although the chemical composition is very similar, small variations can have an impact on the biological activity of the essential oils, and particularly the minor components with synergistic effects with major ones (Lima Oliveira et al., 2018). Further comparison of our results with literature data was not possible due to the different origin of vegetal matrix, AOC assays procedures and expression of results. However, the values obtained were comparable with those usually reported for essential oils (Ruiz-Navajas et al., 2013; Wang et al., 2017; Yashin et al., 2017).

The tested *in vitro* anti-*Salmonella* activity was quite interesting, even considering only the serovars singularly. The antimicrobial activity of coriander seed essential oils with similar (s)-(+)-linalool content has been previously investigated by different authors (Burdock and Carabin, 2009). In the study of Ildiz et al. (2018), the growth inhibition of several bacterial species, including *S.* Typhi, through the use of CEO at different concentrations (1,250–5,000 $\mu g\ mL^{-1}$), was described. Moreover, Delaquis et al. (2002) showed antimicrobial activity of CEO able to inhibit *L. monocytogenes* and *S. aureus*, although without efficacy against *P. fragi* and *S.* Typhimurium. Although the Gram negative-bacteria seem to be more tolerant to essential oils treatment than Gram-positive ones, due to the lipopolysaccharides that protect them from hydrophobic compounds (Hyldgaard et al., 2012), the CEO primary mechanism of action is membrane damage, similarly in both Gram-positive and Gram-negative bacteria (Silva et al., 2011).

The higher sensitivity of *Salmonella* cocktails to the CEO treatment with respect to the single cultures might be due to hormesis phenomenon, nevertheless also the antagonism or competition established between the different cultures should be considered. Probably, to recover the stress caused by the oil, the microorganisms compete for nutrients or energy, hindering each other. Thanissery and Smith (2014) also observed this *Salmonella* behavior when 3 different serovars (Heidelberg, Montevideo, and Enteritidis) were treated with rosemary and clove essential oils in single and mixed culture.

The prolonged Lag period of *Salmonella* cocktails and reduced growth rate in presence of CEO are compatible with cell response to stressing events and represent a measure of the stress suffered by the cells. The cell in this phase in fact recovers a physiological state that allows the start over of multiplication. The speed of recovery of this condition corresponds to the extension of the Lag phase, depending on the time necessary for the different metabolic processes required (Serio et al., 2010). In our case the stressful event was the exposure to sub-lethal concentrations of CEO.

In addition to successful results obtained from *in vitro* studies, coriander essential oil underlined good potential as novel sustainable washing agent against *S. enterica* on carrot samples. This study, in fact, demonstrated that the application of CEO ($5\ \mu L\ mL^{-1}$) for a short time (2 min) effectively decreased *Salmonella* populations in artificially inoculated stick carrots up to 1 day of storage time. However, at the end of the storage period the populations slightly increased on the treated sample, with counts comparable with those of the control. These results, in addition to highlighting the capability of *Salmonella* spp. to survive and growth at low temperature, also evidence the ability of *Salmonella* to adapt to the stress conditions caused by the CEO. In this context, Kalily et al. (2017) demonstrated the adaptation of *S.* Senftenberg to linalool, which also conferred better protection to other antimicrobial treatments. In contrast with our findings, Ndoti-Nembe et al. (2015) in a study conducted on refrigerated mini-carrots, observed no significant reduction of the *S.* Typhimurium load at day 1 of treatment with savory EO (0.35% v/v) while a reduction of about 2 Log CFU g^{-1} was shown at day 9 of this treatment.

Although CEO was not effective during the entire examined storage time, the obtained results suggest that this extract could be used in combination with other natural substances with synergetic effects or in carrier systems (e.g., microemulsions, liposome).

Moreover, the stick carrots treatment with CEO washing solution did not cause any adverse effects on the organoleptic properties of the stick carrots. This factor should not be underestimated since the application of plant derivates, especially essential oils, usually interfere with the product aroma and taste (Lv et al., 2011). Although applied at low concentration, in fact, the intense aroma of essential oils has negative impact on food organoleptic properties, often exceeding the consumers' thresholds of acceptability (Hyldgaard et al., 2012).

Furthermore, carrot color was positively affected by this treatment. This last parameter is an important quality attribute. In particular, cut products are subjected to increased exposed surface area, that can lead to an increase in tissues respiration and subsequent protein, lipids and carbohydrates degradation (Xylia et al., 2018). The color preserving activity should be ascribed to phenolic compounds and to their related antioxidant capacity. These antioxidants, in fact, are well-known compounds utilized to prevent, retard or delay oxidative reaction and increase color stability (Mandal and Mandal, 2015).

CONCLUSIONS

To our knowledge, this is the first report on anti-*Salmonella* activity of *C. sativum* seeds essential oil on carrots. Thus, our findings contribute to enrich the knowledge on the antimicrobial activity of this essential oil against *Salmonella* spp. and on its potentiality as washing treatment in the control of *S. enterica* on fresh cut carrots. The *in vitro* study underlined a positive antimicrobial activity against the selected strains of different serovars, both tested singularly and in cocktails. The *in situ* study allowed to confirm the effectiveness of the essential oil in real matrix, where good sensory evaluation results were obtained upon the utilization of this washing treatment. The chemical investigations allowed to obtain interesting results in terms of total phenolic content and related antioxidant activity that probably contributed to the color stability observed in carrot products.

Future studies should be addressed to optimize the upstream activity, enhancing the essential oil permeability in cells by

ameliorating vehicle systems (e.g., microemulsions, liposome) or by coupling this treatment with another one (e.g., physical treatments) to extend its efficacy during time.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors (AR and AS) upon reasonable request.

AUTHOR CONTRIBUTIONS

MP: *C. sativum* seeds derivates extraction, chemical characterization, and draft preparation. CR: microbiological analyses and draft preparation. SP: support in chemical characterization. FM: support in microbiological analyses. CC-L and CL: data analyses. AP: experimental idea. DD: strains provision. AR and AS: data discussion and manuscript revision.

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